

PATENT APPLICATION

**METHOD OF PREPARING BIOLOGICAL MATERIALS FOR
CRYOPRESERVATION USING PRE-CHILLED PROTECTANT**

ABSTRACT OF THE DISCLOSURE

A method of preparing biological materials for cryopreservation is presented.

5 The method lessens the amount of heat released by a cryoprotectant during a latent heat phase by freezing the protectant, thawing the protectant, and treating biologically active materials with the thawed protectant. First, the protectant is frozen to induce an irreversible phase change, along with an irreversible release of energy. After this phase change has occurred, the protectant is thawed and used to treat viable cells or other biologically active material about to undergo freezing. The thawed protectant within the biologically active cells has a reduced endothermic reaction upon subsequent freezing. In one embodiment, freezing the biological material includes immersing the biological material in cooling fluid and circulating the cooling fluid past the biological material at a substantially constant, pre-determined velocity and temperature such that the biological material is vitrified, thus minimizing the formation of stress fractures in cell membranes. The protectant may be propylene glycol, glycerol, DMSO, or other suitable protectants.

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